

To Study Anthelmintic Actiity of Aegle Marmalos

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Abstracts: -

Soil transmitted helminthiases and anthelmintic resistance pose major concern to the human and animal health. Therefore, there is a dire need to identify new sources of anthelmintic drug molecules. One such source could be herbal drugs commonly used to prepare herbal medicine.

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I. INTRODUCTION

1. Gastro intestinal parasites create a serious threat to the production of livestock in developing nations. Anthelmintics are those agents that expel parasitic worm (helminthes) from the body, by either stunning or killing them. Helminthes parasite infections are global problems with severe social and economic repercussions in the third world countries. The diseases affect the health status of a large fraction of human population as well as animals. Some type of dangerous helminthes infections like filariasis has only a few therapeutic modalities at present. Helminthes infections are commonly found in community and being recognized as cause of much acute as well as cattle's use of herbs could be one of the major options to control these pathologies. The literature survey reveals that Sophora interrupta is used to treat various types of gastrointestinal problems. Therefore, an attempt has been made to evaluate anthelmintic activity of leaves on adult earthworm *Pheritima posthuman*. *Sophora interrupta* belongs to the family Fabaceae is commonly called as *Edwardia maderaspatana* Wight, *Pili Grigol*. There are approximately 219 species in genus *Sophora*.

2. The NPGS distributes plant germplasm to professional plant breeders and other career research scientists. Requests from producers will be considered individually, when NPGS germplasm is not readily available from commercial sources. Requests for educational purposes are usually considered only from college/university faculty and students when NPGS material is integral to the project. Educational requests submitted by students should be accompanied by an endorsement from a sponsoring faculty. Any requestor may be required to provide additional information about their order, including a detailed explanation of intended purpose and proof of affiliation or professional status. Please view the short video on the NPGS for more details. *Aegle marmelos* (family: Rutaceae)

3. *Aegle marmelos* L. (Family: Rutaceae) commonly known as *Bael* in Hindi is an essential food plant of India. Traditionally the fruit was used to treat diabetes, respiratory problem, inflammation, dysentery and diarrhea. The fruits of *Aegle marmelos* are rich in flavonoids, terpenoids, carotenoids and coumarins. The major bioactive constituents include imperatorin (54), aegelin, lupeol, eugenol (7), cineol, citronellal etc. The CYP450-CO assay to evaluate the inhibitory potential of the fruit extract and the standard phytoconstituent imperatorin revealed dose dependent inhibition of the *Aegle marmelos* extract.

4. *Aegle marmelos* Correa commonly known as *Belva* or *Sri phal* or *Shivendra* (the tree of shiva) in Sanskrit, *Bel* or *Bael* in Hindi, and as *wood apple*, *stone apple*, *Bengal quince*, *Indian quince*, *holy fruit* or *golden apple* in English, is an important medicinal tree in India. *Bael* trees are indigenous to India and are found growing in abundance in the Himalayan regions, Bengal, Central and South India, as well as in Sri Lanka, Burma, Thailand, Bangladesh, Nepal, Vietnam, Laos, Cambodia, and Pakistan. *Bael* leaves are offered to the Indian deity, Lord Shiva, and the tree is planted extensively in temples for this reason (Das and Das, 1995; Malty et al., 2009).

5. The leaf is trifoliate, alternate, each leaflet 5–14 cm (2–5+1/2 in) x 2–6 cm (3/4–2+1/4 in), ovate with tapering or pointed tip and rounded base, untoothed or with shallow rounded teeth. Young leaves are pale green or pinkish, finely hairy while mature leaves are dark green and completely smooth. Each leaf has 4–12 pairs of side veins which are joined at the margin. Collection, authentication and extraction of *A. marmelos* leaves

II. Materials & method

Collection & authentication of plant aegle marmelos

Collection, authentication and extraction of *A. marmelos* Collection of *A. marmelos* leaves was undertaken from areas in and around Chandigarh, India during the month of January. Dr. Sujata Bhattacharya, Assistant Professor, School of Biological and Environmental Sciences, Shaolin University, Solan authenticated the plant material. Voucher specimens of the plant (SUBMS/89) were deposited in the School of Biological and Environmental Sciences, Shaolin University, Solan. The dried coarsely powdered leaves of the plant (500 g) were first extracted with the petroleum ether followed by 70% ethanol by the hot extraction process using a Soxhlet apparatus [17, 18]. The solvent removed by distillation under reduced pressure after completion of extraction process and the prepared extract was stored in vacuum desiccator until further use.

3) phytochemical Screening

Phytochemical screenings were performed using standard procedures as follows

Reducing Test Sugars (Fehling' test) The aqueous ethanol extract (0.5 g in 5 ml of water) of individual plants was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

Anthraquinones Test for the individual plant extract (0.5 g) was boiled with 10 ml of sulphuric acid (H₂SO₄) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for Terpenoids (Salkowski test) To 0.5 g each of the individual extract was added 2 ml of chloroform. Concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown coloration was confirmed for the presence of terpenoids.

Test for Flavonoids A portion of the individual plant extract (0.5 g) was heated with 10 ml of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

Test for Saponins To 0.5 g of each plant extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for Tannins About 0.5 g of the individual extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1 % ferric chloride (FeCl₃) was added and observed for brownish green or a blue-black coloration.

Test for Alkaloids 0.5 g of each extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloid base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and drageoir's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish-brown precipitate (with Drageoir's reagent) was regarded as positive for the presence of alkaloids.

Test for Cardiac Glycosides (Keller-Killian test) To 0.5 g of individual plant extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under laid with 1 ml of concentrated H₂SO₄. A brown ring at the interface indicated the presence of a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Animals

Phreesia

posthuman (Adult Indian earth worms) of about 5-7 cm long were used for the present study.

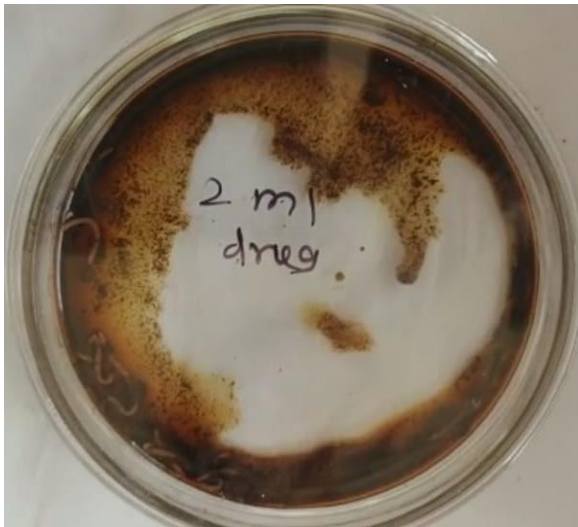
Standard Drug used

Albendazole suspension (micronized albendazole suspension in the concentration of 10 mg / ml) was used as the standard to compare the test resell

Anthelmintic Activity: - Phreesia posthuman (Indian adult earth worms) of nearly equal size (6 CMS ± 1) were selected randomly for the present study¹⁰⁻¹². The worms were acclimatized to the laboratory conditions before experimentation. The earth worms were divided into four groups of six earth worms in each. Albendazole suspension in the concentration of 10 mg / ml served as a standard and poured into petri dishes. The test extract were prepared in the concentrations of 5 mg / ml, 10mg / ml, 15 mg / ml, 20 mg / ml, 25 mg / ml, 30 mg / ml. Normal saline served as control. Six earth worms nearly equal size 6 CMS ± 1 were taken for each concentration and placed in petri dishes at room temperature¹³. The time taken for complete paralysis and death were recorded. The mean paralysis time and mean lethal time for each sample was calculated. The time taken for the worms to become motionless was noted as paralysis time and to ascertain death, each worm was frequently applied with external stimuli which stimulates or induce movements in the earthworm if alive

- Treatment Concentration on used (mg/ml) Time taken for paralysis (min) X Time taken for death (min)

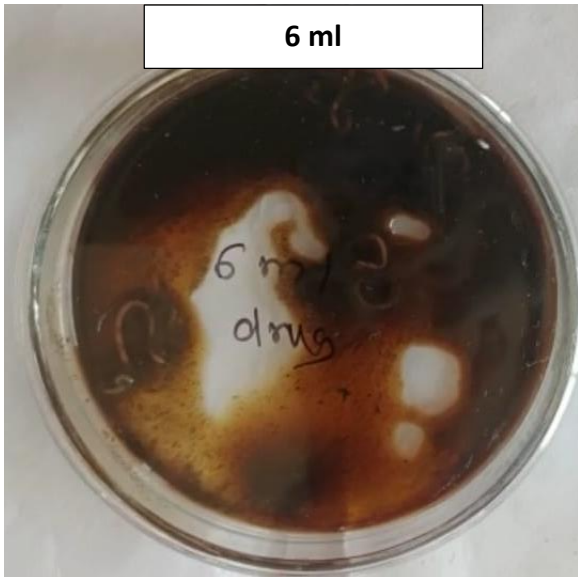
2 ml



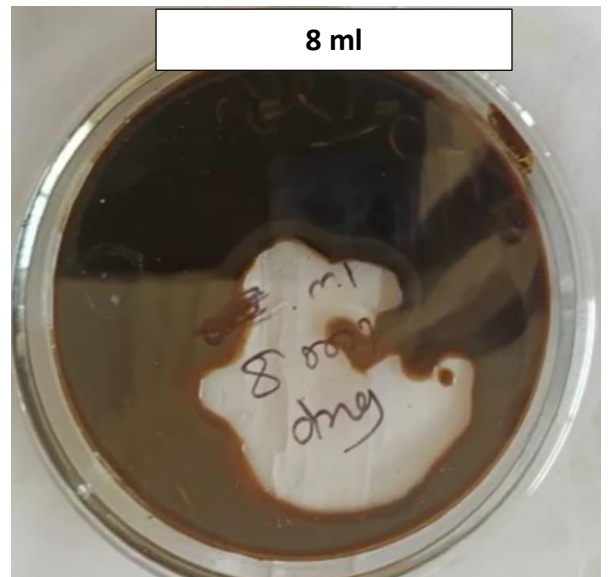
4 ml



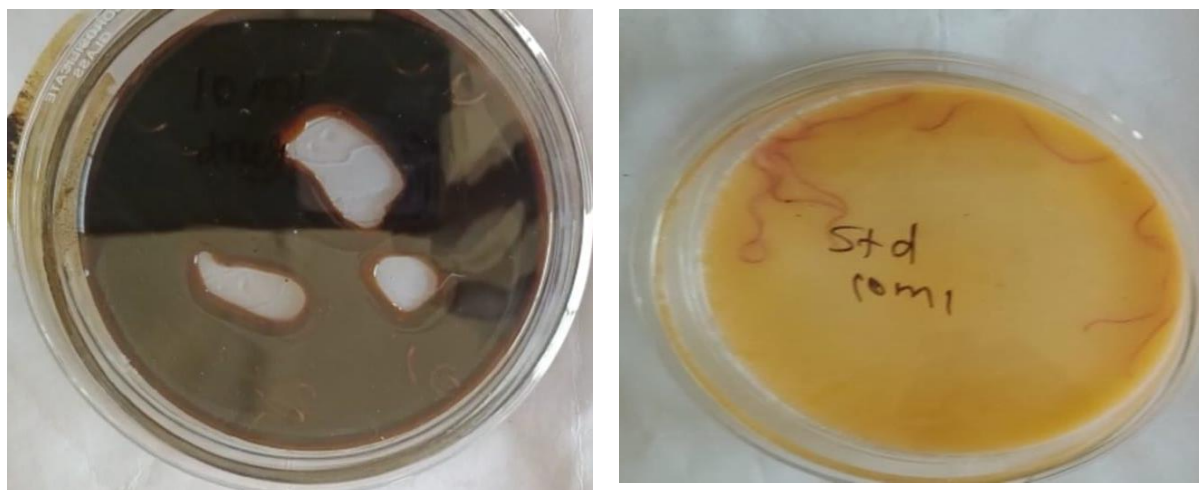
6 ml



8 ml



Std 10 ml



Treatment	Concentration used (mg/ml)	Timetaken for paralysis (min) X ± S.D	Time taken for death (min) X ± S.D
Control	---	---	---
Standard (Albendazole)	10 mg / ml	17 ± 1.571*	39 ± 1.932*
Methanolic extract of <i>Sophora interrupta</i>	5 mg / ml	76 ± 3.303*	99.33 ± 0.402*
	10 mg / ml	65.33 ± 2.883**	90.67 ± 3.921**
	15 mg / ml	56 ± 2.017**	85.00 ± 5.310**
	20 mg / ml	4.33 ± 1.498**	69.83 ± 2.496**
	25 mg / ml	48.33 ± 1.498**	51.67 ± 2.108**
	30 mg / ml	27.33 ± 2.060**	40.33 ± 0.9888***

All values are Mean ± SEM analyzed by one way ANOVA followed by Dunnett's test. *p < 0.05, ** p < 0.01, *** p < 0.001

III. DISCUSSION

Some of the traditionally used herbs have scientifically proved a potent anthelmintic activity by using suitable experimental models. The predominant effect of Albendazole on the worm is to cause flaccid paralysis that result in expulsion of the worm by peristalsis. Albendazole by increasing chloride ion conductance of worm muscle membrane produces hyperpolarization ad reduced excitability that leads to muscle relaxation and flaccid paralysis.

The extract demonstrated paralysis as well as death of the worms at a time comparable to Albendazole especially at higher concentration of 30 mg / ml. Poly phenolic compounds shown anthelmintic activity; chemically tannins are poly phenolic compounds. Some synthetic phenolic anthelmintics, e.g.: - Nicodamid, Oxyclozanide and Bithionol are shown to interface with energy generation in helminth parasites by uncoupling oxidative phosphorylation. It is possible that the active

IV. Conclusion

Aegle marmelos leaf extracts significantly inhibit the growth of all dermatophyte's fungi studied. If this activity is confirmed by in vivo studies and if the compound is isolated and identified, it could be a remedy for dermatophytosis. Principle like tannins in the extract of *Sophora interrupta* produces similar effects. Another possible anthelmintic effect of tannins is that they can bind to free protein in the gastro intestinal tract of host animal or glycol protein on the parasite and cause death¹⁵. Further the extract can be tested on various other helminthes to ascertain the anthelmintic activity on a broader scale which is our future plan of research work.

V. Result

Aegle marmelos leaf extracts and fractions were found to have fungicidal activity against various clinical isolates of dermatophytid fungi. The MIC and MFC was found to be high in water and ethyl alcohol extracts and methanol fractions (200µg/mL) against dermatophyte fungi studied.

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